

## PROTOCOL



# The comparison of enamel hardness between fluoride and theobromine application

Abdillah Imron Nasution, Cut Zawil

Department Oral Biology, Faculty of Dentistry, Syiah Kuala University, Kopelma Darussalam, Kota Banda Aceh, Aceh-Indonesia

### Correspondence

Abdillah Imron Nasution, Department Oral Biology, Faculty of Dentistry, Syiah Kuala University, Kopelma Darussalam, Kota Banda Aceh, Aceh-Indonesia.  
E-mail: [abdillah@karstaceh.com](mailto:abdillah@karstaceh.com)

Received 01 December 2014;

Accepted 29 December 2014

doi: 10.15713/ins.ijcdmr.14

### How to cite the article:

Abdillah Imron Nasution, Cut Zawil, "The comparison of enamel hardness between fluoride and theobromine application," *Int J Contemp Dent Med Rev*, vol.2014, Article ID 031214, 2014.  
doi: 10.15713/ins.ijcdmr.14

### Abstract

**Background:** The application of fluoride as preventing caries still raises the pros and contras among scientists. Theobromine that is contained in cocoa is potentially element to preventing caries. Therefore, no data about differences hardness in the enamel surface using standard fluoride application and theobromine. **Objective:** The aim of this study is to analyze the differences hardness between fluoride and theobromine application on enamel surfaces. **Materials and Methods:** This study compares values before and after intervention in enamel was conducted in June 2013 at the Faculty of Mechanical Engineering University of Syiah Kuala. Specimens were 42 premolars collected from extracted tooth for orthodontic treatment in Banda Aceh. Specimens divided into two groups: Theobromine 2% and fluoride 2%. The hardness measurements calculated by Shimadzu HMV2 Hardness Tester. Data analyzed by paired sample t-test Microsoft Excel Analyse-it version 3.20. **Result:** The hardness number in Fluorosis-before and after was 321.33 Vickers hardness number (VHN) versus 355.48 VHN. The hardness number in theobromine before and after was 319.99 VHN versus 341.14 VHN. The comparison test showed significant level ( $P < 0.05$ ). **Conclusion:** application of fluoride and theobromine are equally able to increase the hardness of the enamel surface. Furthermore, the hardness of the enamel surface by fluor application is higher than the theobromine.

**Keywords:** Enamel fluoride, theobromine, Vickers hardness number

### Introduction

Dental caries is the most common infectious disease affecting humans. The principal causative agents are a group of streptococcal species that *Streptococcus mutans* are the most important agents of human caries.<sup>[1]</sup> According to Kidd (1992) there is four factors that responsible for dental caries, namely microorganisms, substrates, host, as well as time. To be noted, all these factors must work simultaneously.<sup>[2]</sup> Dental caries has become a disease that spread through the world, at almost 95% of the world's population suffers from dental caries while the incidence of dental caries in Indonesia reached at relatively high rate, i.e., 90.05%.<sup>[3]</sup> In order to treat this problem, required precautions can be done by effective, efficient, and affordable caries prevention methods and materials.

The most successful caries-preventive agent is fluoride. Fluoride improves the biological apatite and the caries resistance of the teeth. However, the systemic fluoride administration was necessary for maximum benefit. Now-a-days, caries reduction, therefore, has to be balanced against increasing dental fluorosis.

The caries resistance concept was shown to be erroneous 25 years ago, but the new paradigm is not yet fully adopted in public health dentistry, thus scientist still await real breakthroughs in more effective use of fluoride for caries prevention.

Fluor is an important element in the formation of teeth and bones. Fluor joined with other elements forming the compounds of fluoride. Fluoride compounds were used in the prevention of dental caries at long time ago. Effectiveness of fluoride has been proven enhance the process of tooth remineralization and widely used commercially by the public and dentists in the form of toothpaste, mouthwash, and even chewing gum.<sup>[4]</sup> In improving dental health, fluoride compounds have been extensively applied, and its effectiveness has been recognized. The use of compounds of fluoride can be done systemically, The American Dental Hygienist's Association recommends the use of 1 ppm fluoride in drinking water,<sup>[5]</sup> whereas according to the American Dental Association the safety level of topical application of sodium fluoride is a 2%, stannous fluoride 8%, and acidulated phosphate fluoride is 1.23%.<sup>[6,7]</sup>

Furthermore, fluoride is widely used in caries prevention. In fact, the application of fluoride still raises the pros and contras among scientists. Other element that can be used preventing caries is theobromine that is contained in cocoa. The main reason of this debate is if fluoride not restricted will harm the tooth, named fluorosis.<sup>[8]</sup> Whereas, the excessive use of theobromine application will cause nothing. Sadeghpour, (2007) stated that the cocoa extract is more effective than fluoride in reducing dental caries in animal model. Same as fluoride, theobromine can prevent demineralization and improve resilience of tooth by acid.<sup>[9]</sup>

In 2002, Nakamoto proved that the theobromine can increase the size of apatite crystals and increase resistance of tooth to acid dissolution.<sup>[10]</sup> Previous research stated by the microhardness values, a consistent and remarkable protection of the enamel surface was found with the application of theobromine 200 mg/l.<sup>[11]</sup> The conclusion is fluoride and theobromine increased resistance of enamel to acid dissolution as well as influence the hardness of enamel surface.

Therefore, no data about differences hardness in the enamel surface using standard fluoride application and theobromine as the potential prevention of caries. The hardness is one of the mechanical properties of the material. One of the tools for measuring the surface hardness is by using micro-Vickers hardness test. Based on the previous report, the Vickers hardness number (VHN) of enamel hardness was varied. Graspersic report enamel hardness in occlusal is 359.5 to 424.3 VHN.<sup>[12]</sup> Ryge reported enamel hardness ranges from 254 to 348 VHN, while Reyes-Gasga reported rates of enamel hardness is 254-383 VHN.<sup>[13]</sup> Based on the problems, the aim of this study is to analyze the differences hardness between fluoride and theobromine application on enamel surfaces as the alternative caries prevention.

## Materials and Methods

This paired study compare values before and after intervention in enamel was conducted in June 2013 at the Laboratory of the Department of Dental Materials Dentistry University of Indonesia. Specimens were 42 premolars that collected from extracted tooth for orthodontic treatment from various dentistry clinics in Banda Aceh. Specimens were free from caries, abrasion, and fluorosis. Specimens collected in 6 month (1 October 2012-April 2013). Sample size determination by equation:

$2 + C \left( \frac{s}{d} \right)^2$  where  $s$  is the standard deviation,  $d$  is the difference to be detected, and  $C$  is a constant dependent on the value of  $\alpha$  and  $\beta$  selected.  $C$  can be determined from two levels of  $\alpha$  and  $\beta$ . Note that for  $\alpha = 0.05$  and  $1 - \beta = 0.9$ ,  $C$  is 10.51 if  $s$  is 3,  $d$  is 2, then each group is 21 specimens. Cocoa powder collected from farmer in Aceh-Indonesia as much as 0.2 g dissolved in 1 L aquades to obtain a concentration of 2% and Fluoride as much as 0.2 g powder was dissolved in 1 L aquades to obtain a concentration

of 2%. All specimens divided randomly into two groups theobromine 2% and fluoride 2%. The specimens were cut at the cervical part to separate the crown and roots. Specimens were then embedded in acrylic molds with a diameter of 5 mm and a height of 2 mm; with the buccal tooth surfaces are parallel to the diameter of the mold. The buccal of enamel surface was grinding by silicon carbide no. 1000 and 1200 and placed in the grinding and polishing machine. Controlling the loss of enamel surfaces were by microscope observation. Enamel polished with 0.2 and 0.5  $\mu$  alumina liquids and washed with aquades.<sup>[14]</sup> Specimens were separated and marked based on the group.

Both groups immersed in distilled water for data before and Group 1 was immersing in theobromine 2% and Group 2 in fluoride 2% for data after. Immersed time is 5 min with a frequency of treatment as much as 24 times. Immersed conducted by assuming 5 min is the minimum time to consume the chocolate, and hence the amount of consumption for 6 months is 24 times.<sup>[4,7]</sup> Furthermore, the surface hardness measurements were taken and recorded. Enamel surface hardness measurements using Shimadzu HVM2 Hardness Tester. Each specimen was measured three times to have the average hardness numbers. Analysis of hardness number conducted by paired  $t$ -test analysis in Microsoft Excel Analyze-it version 3.20.

## Results

The normality data test using Shapiro-Wilk showed all data normal ( $P > 0.05$ ). The mean of hardness number in fluoride-before was 321.33 VHN (range: 320-323 VHN) and after was 355.48 VHN (range: 351-358 VHN). The mean of hardness number in theobromine before was 319.99 VHN (range: 319-321 VHN) and after was 341.14 VHN (Range: 340-342 VHN). Result showed fluoride groups have higher hardness number than theobromine group. The comparison test by  $t$ -test study between theobromine and fluoride showed significant level ( $P < 0.05$ ). The hardness number of each group can be seen in Table 1.

## Discussion

Based on research results obtained those surface hardness numbers were varies in enamel. Each group showed an increase in the value of surface hardness number of enamel after treatment. These results indicate that the fluoride have better improve the hardness of the enamel surface compared with the control and theobromine.

Enamel was structured by enamel rod that formed by crystal apatite.<sup>[14]</sup> Apatites have the general formula,  $\text{Ca}_{10}(\text{PO}_4)_6\text{X}_2$  where  $X$  is typically fluor (fluorapatite [FA]), hydroxyapatite (OH), or chlorapatite (Cl).<sup>[15,16]</sup> The apatite lattice is very tolerant by substitutions, vacancies, and solid solution. Thus, the element of OH in the structure can be substituted by the fluor.<sup>[17]</sup> There is an additional element of fluoride on enamel causing

**Table 1:** The enamel hardness number of each group

No	Theobromine (VHN)		Fluoride (VHN)	
	Before	After	Before	After
1	320	340	321	358
2	320.3	341	320	354
3	321	341	321	351
4	321	341	321	355
5	320	340	321	357
6	320	341	321	355
7	320.6	341	322	357
8	319	341	320	354
9	320	342	320	354
10	321	342	321	357
11	319	341	321	354
12	320	340	320	355
13	320	341	321	357
14	320.2	341	322	357
15	320	341	322	356
16	320.1	342	323	355
17	319	341	321	355
18	319.7	342	322	354
19	320.2	341	322	358
20	319	342	323	357
21	319.7	342	323	355
	319.99	341.14	321.33	355.48

VHN: Vickers hardness number

substitution of hydroxyl ions (OH) by Fluor ions to forming FA.<sup>[17]</sup> There are two types of interaction with the fluorapatite. Fluor, join in the crystal lattice structure and bound to the crystal surface. Fluor ion has the ability to diffuse through the hydration shell that surrounds each crystal of HA as a result fluoride can replace the OH group on the surface. Furthermore, fluor ions can migrate into the crystal body and become one with the framework of the interior lattice of hydroxy apatite. Fluor that enters the crystal lattice can cause changes in shape and physical properties of crystals.

Fluor will replace the OH group on the surface of the crystal, but not all the OH replaced with Fluor, consequently formed hydroxy FA.<sup>[17]</sup> Donadel<sup>[18]</sup> reported the enamel structure, which formed by FA demonstrate different structure than HA. Most of fluoride will diffuse back out and a small portion settled and reacts with HA to form several chemical compounds depending on the condition.<sup>[19]</sup> Both interactions play a significant role in the process of apatite solubility.

OH is an element contains water and easily dissolved and fluor ions that replacing OH known as gaseous atom. In the periodic table shows that the position of the F atom is more to the right compared with O and H and hence that the ionization

energies are generally more greater.<sup>[16]</sup> The ionization energy is required to remove electrons from an atom. The ionization energy value of O is 1362 kJ/mol and H is 1360 kJ/mol, whereas the value of Fluor is more greater (1742 kJ/mol).<sup>[16,19]</sup>

In c-axis the position of ion Fluor is higher than the position of OH ions thus Fluor can increase the stability of the apatite structure. Consequently, Fluor is more stable than OH to form a stronger structure.<sup>[12]</sup> According calderyn,<sup>[20]</sup> OH position on the c-axis is between  $\frac{1}{4}$  and  $\frac{1}{3}$  c-axis, while the position of F ions in the upper triangle is  $\frac{1}{4}$  of central of Ca atoms. The position of F ions is higher resulting in elongation of the cell unit of c-axis of apatite. This phenomenon indicated as attempting stability of apatite crystal to decrease of interatomic bonding and minimalism the effect of microstrain in apatite crystal atoms.<sup>[17]</sup>

The theobromine analyzed showed an increase in surface hardness of the enamel surface. theobromine can increase the surface hardness of enamel by the reaction of interstitials. William stated in cell unit of apatite crystal is micro tunnel with a diameter  $\pm 176$  pm. It possible to smaller ion can pass the tunnel to generating the interstitial reaction of theobromine ions on apatite crystals. The molecular formula for theobromine is  $C_7H_8N_4O_2$ .<sup>[21]</sup> Some ions in theobromine (C = 170 pm, N = 152 pm, and H = 152 pm) have smaller than the diameter of micro tunnel.<sup>[17]</sup> The substitution of other ions on apatite crystal will change the physical properties of apatite itself. The dense arrangement of apatite crystals will reduce or minimize the appearance of forces between adjacent atoms. Consequently, it has required greater force to separate all atoms that arranged the crystal.<sup>[17]</sup> Indirectly this will increase the density of apatite crystal and make it more difficult to break. In the macroscopic case is seen as an increasing of enamel surface hardness.<sup>[11,17]</sup>

Based on the results is concluded that the application of fluoride and theobromine are equally able to increase the hardness of the enamel surface. Furthermore, the hardness of the enamel surface by fluor application is higher than the theobromine.

## Acknowledgments

The author would like to thank all the undergraduate students of Faculty of Dentistry, University of Syiah Kuala, Aceh-Indonesia for their participation in this study.

## References

- Balakrishnan M, Simmonds RS, Tagg JR. Dental caries is a preventable infectious disease. *Aust Dent J* 2000;45:235-45.
- Kidd EA, Fejerskov O. Dental Caries: The Disease and Its Clinical Management. Oxford: Blackwell Munksgaard; p. 50-69.
- NIHRD: Indonesia: Sub-national health system performance assessment. Jakarta: National Institute of Health Research and Development (NIHRD), Ministry of Health, Republic of Indonesia; 2005. WA 546 IND; 2005.
- Chen H, Czajka-Jakubowska A, Spencer NJ, Mansfield JF, Robinson C, Clarkson BH. Effects of systemic fluoride and *in vitro*

- fluoride treatment on enamel crystals. *J Dent Res* 2006;85:1042-5.
5. Fluoride Database of Selected Beverages and Foods. United States: Department of Agriculture; 2005. Available from: <http://www.ars.usda.gov.1>.
6. Harris NO, Godoy FG. Primary Preventive Dentistry. 6th ed. New Jersey: Prentice Hall Health; 2004. p. 248-9.
7. Avery DR, McDonald RE. Dentistry for the Child and Adolescent. 7th ed. St. Louis: Mosby; p. 229-36.
8. Aoba T, Fejerskov O. Dental fluorosis: chemistry and biology. *Crit Rev Oral Biol Med* 2002;13:155-70.
9. Sadeghpour A. Fight tooth decay with chocolate, reveals study. *Br Dent J* 2007;202:648.
10. Nakamoto T. Theobromine Increases Crystal Size. Available from: [http://www.iadr.confex.com/iadr/2002.SanDiego/techprogram/abstract\\_12740.htm](http://www.iadr.confex.com/iadr/2002.SanDiego/techprogram/abstract_12740.htm).
11. Kargul B, Özcan M, Peker S, Nakamoto T, Simmons WB, Falster AU. Evaluation of human enamel surfaces treated with theobromine: a pilot study. *Oral Health Prev Dent* 2012;10:275-82.
12. Gaspersic D. Enamel microhardness and histological features of composite enamel pearls of different size. *J Oral Pathol Med* 1995;24:153-8.
13. Reyes-Gasga J, Gutiérrez-Salzar MP. Enamel hardness and caries susceptibility in human teeth. *Rev Latin Am Met Mat* 2001;21:1-10.
14. Wongkhantee S, Patanapiradej V, Maneenut C, Tantbirojn D. Effect of acidic and drinks on surface hardness of enamel, dentine, and tooth-coloured filling materials. *J Dent* 2005;10:1-7.
15. Mathai M, Shozo T. Structures of biological minerals in dental research. *J Res Natl Inst Stand Technol* 2001;106:1035-44.
16. Elliott JC. Structure and Chemistry of the Apatites and Other Calcium Orthophosphates. Amsterdam: Elsevier; 1994.
17. Gunawan HA. The effect of changes in apatit, level retention and intrusion fluorine on solubility. Email after Treatment *S. insularis*. Jakarta: Dissertation. Faculty of Dentistry, University of Indonesia; 2006. p. 53-106.
18. Donadel K, Laranjeira MC, Goncalves VL, F'avere VT, Machado KD, *et al*. Structural, vibrational, and mechanical studies of hydroxyapatite produced by wet-chemical methods. *arXiv*: 2004; physic: 0402002 v1.
19. Lide DR. CRC Handbook of Chemistry and Physics. Atomic, Molecular, and Optical Physics; Ionization Potentials of Atoms and Atomic Ions. 84th ed., Sec. 10. Boca Raton, Florida: CRC Press; 2003.
20. Calderyn L, Stott MJ, Rubio A. Electronic and crystallographic structure of apatite. *Phys Rev* 2003;B67:1341-6.
21. Malisoff WM. Dictionary of Bio-Chemistry and Related Subjects. New York: Philosophical Library. p. 311, 530, 573.